

Applicant: Jay Short, *et al.*
Serial No.: 08/876,276
Filed: June 16, 1997
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In the Claims

~~Please cancel~~ claims 1-18 without prejudice.

~~Please add~~ the following new claims:

--19. (New) A method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA comprising:

- stably inserting a bioactive substrate that is fluorescent in the presence of the activity of interest into clones in a library containing a plurality of clones obtained from more than one organism;
- screening the library with a fluorescent analyzer that detects bioactive fluorescence, and
- identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule.--

--20. (New) The method of claim 19, further comprising obtaining DNA from a clone that is positive for an enzymatic activity of interest.--

--21. (New) The method of claim 20, wherein the enzyme is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.--

--22. (New) The method of claim 19, wherein the library is generated in a prokaryotic cell.--

--23. (New) The method of claim 22, wherein the library contains at least about 2×10^6 clones.--

--24. (New) The method of claim 22, wherein the prokaryotic cell is gram negative.--

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--25. (New) The method of claim 19, wherein the clones are encapsulated in a gel microdrop.--

--26. (New) The method of claim 19, wherein the analyzer screens up to about 15 million clones per hour.--

--27. (New) The method of claim 19, wherein the clones are extremophiles.--

--28. (New) The method of claim 27, wherein the extremophiles are thermophiles.--

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--29. (New) The method of claim 27, wherein the extremophiles are hyperthermophiles, psychrophiles, halophiles, psychrotrops, alkalophiles, or acidophiles.--

--30. (New) The method of claim 19, wherein the bioactive substrate comprises staining reagent Cl^{12}FDG .--

--31. (New) The method of claim 19, wherein the bioactive substrate comprises a lipophilic tail.--

--32. (New) The method of claim 19, wherein the clones and substrates are heated to enhance stable insertion of the substrate into the clones.--

--33. (New) The method of claim 32, wherein the heating is to a temperature of about 70°C.--

--34. (New) The method of claim 32, wherein the heating is for about 30 minutes.--

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--35. ✓ (New) The method of claim 19, wherein the fluorescent analyzer comprises a fluorescence activated cell sorting (FACS) apparatus.--

--36. (New) The method of claim 19, wherein the enzyme encoded by the mutagenized DNA is stable at a temperature of at least about 60°C. —
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--37. ✓ (New) The method of claim 19, wherein the library is an expression library.--

--38. (New) The method of claim 19, wherein the enzyme encoded by the DNA possesses enhanced enzymatic activity of interest compared to that of the enzyme encoded by the non-mutagenized DNA. --
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cont

--39. ✓ (New) The method of claim 19, wherein the method further comprises biopanning the expression library prior to stably inserting the substrate. --

--40. (New) The method of claim 19 further comprising obtaining DNA from a clone identified in step c) that is positive for an enzymatic activity of interest and comparing the enzymatic activity of a DNA expression product from the clone with that obtained from such a clone into whose DNA at least one mutation has been introduced, wherein a difference in enzymatic activity is indicative of the effect upon the enzymatic activity of interest caused by introduction of the at least one mutation. --

--41. (New) The method of claim 19, wherein the bioactivity encoded by the DNA possesses the bioactivity of interest at a temperature at least 10°C below the temperature of optimal activity of the bioactivity encoded by the non-mutagenized DNA. --